

# Why is lateral root growth so variable?

## A framework to analyze growth variability among lateral roots and the possible roles of auxin and carbon

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Lateral root (LR) development allows the plant to explore the soil volume and capture water and minerals resources efficiently. In most species, annual or perennials, monocots or dicots, a large intrinsic variability in LR length is present. Such variability could be an adaptive trait enabling a greater plasticity and efficiency of the root system considering the spatial and temporal heterogeneity of soil resources (Forde 2009; Pagès 2011).

While the molecular pathways involved in the development of primordia and young LRs (and in particular the contributions of auxin) are well characterized (eg. Lavenus 2013), we know surprisingly little about the mechanisms and actors involved in the *variability* of LRs. Because root growth rate is dependent on assimilate availability (Freixes 2002), we hypothesized that both auxin fluxes and sugar status of individual LRs could be involved in the variability of LR fate.

We use maize plants as a model and have developped a phenotyping and computational pipeline to aquire and statistically analyse the LR growth patterns. Moreover, we have modified carbon and auxin fluxes to the LR using excision of all roots but the primary root and evaluated auxin and sugar status using biochemical and molecular markers.

### 1 Phenotyping LR growth in maize plants

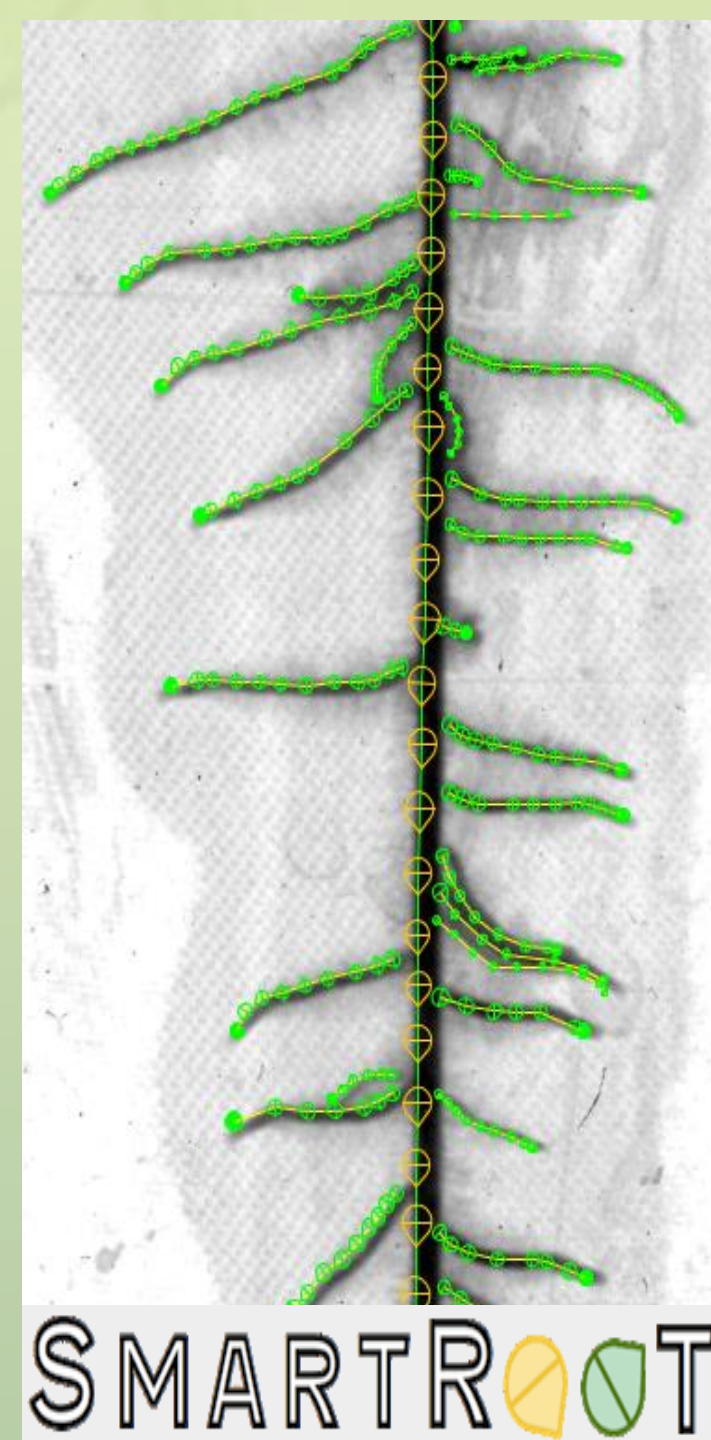


We used a **rhizotron** system (Neufeld 1989) to force the root system of young maize plants to grow in 2D.



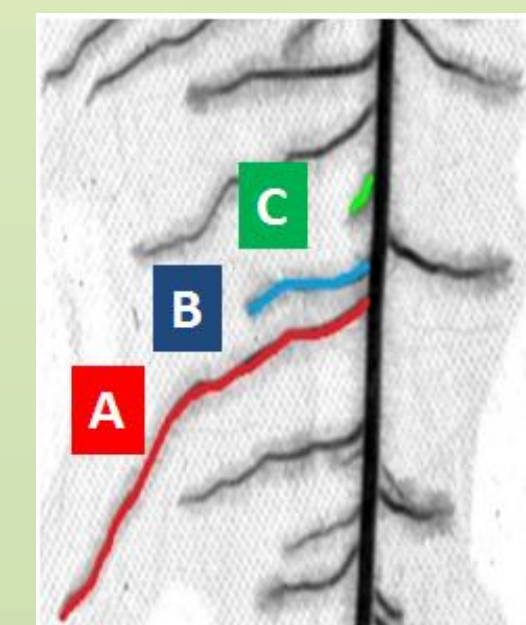
Individual root systems were scanned daily at high resolution (720 dpi) during 15 days.

Morphological and growth traits for each individual root were extracted using **SmartRoot** (Lobet 2011) and analyzed using R software.



### 2 We have identified 3 main LR growth profiles

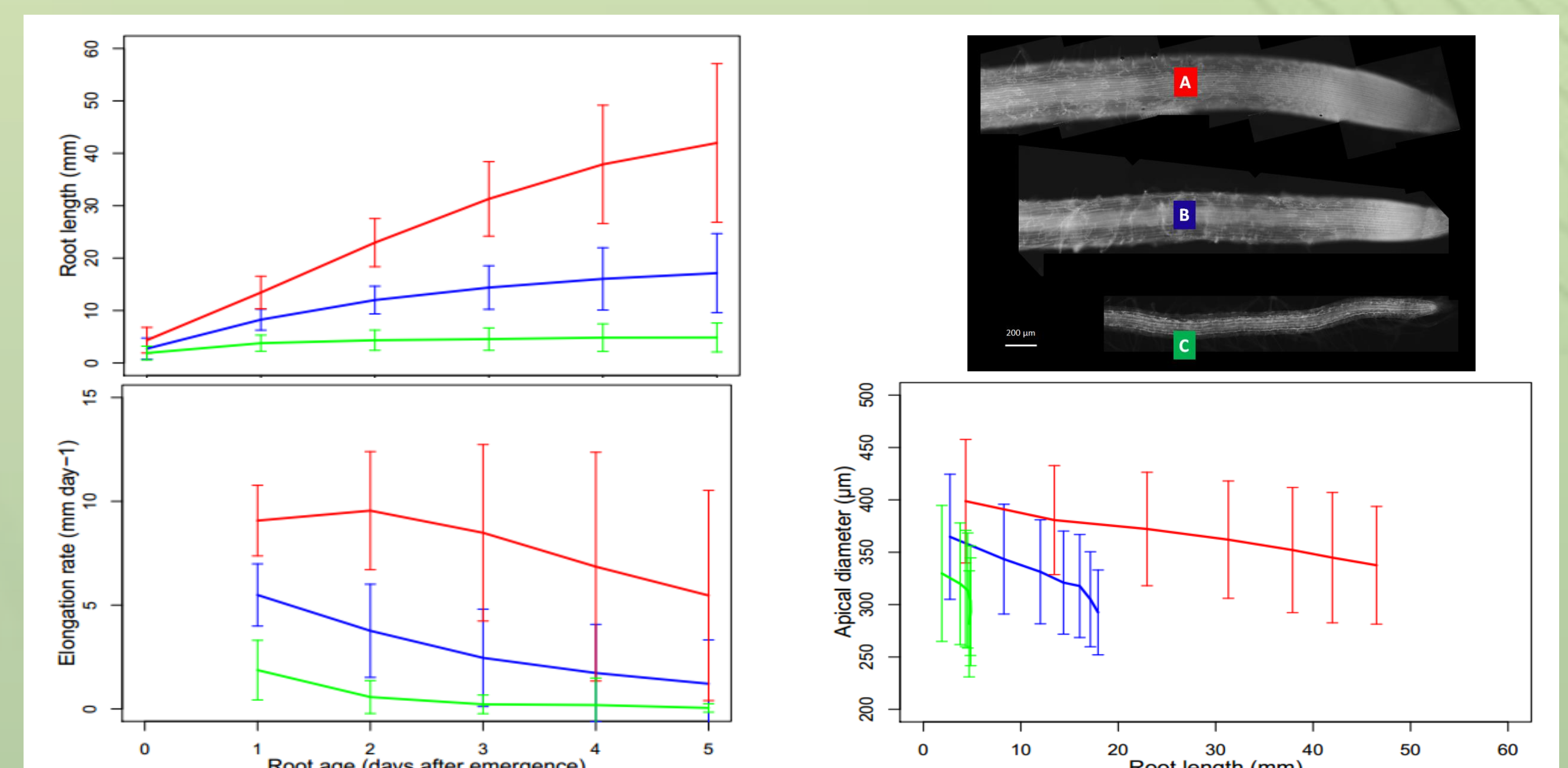
LR length and diameter were highly variable among roots of the same age (i.e. neighbours along the primary root). On the basis of LR lengths at day 1-3 after emergence, we identified 3 main **LR growth profiles**.



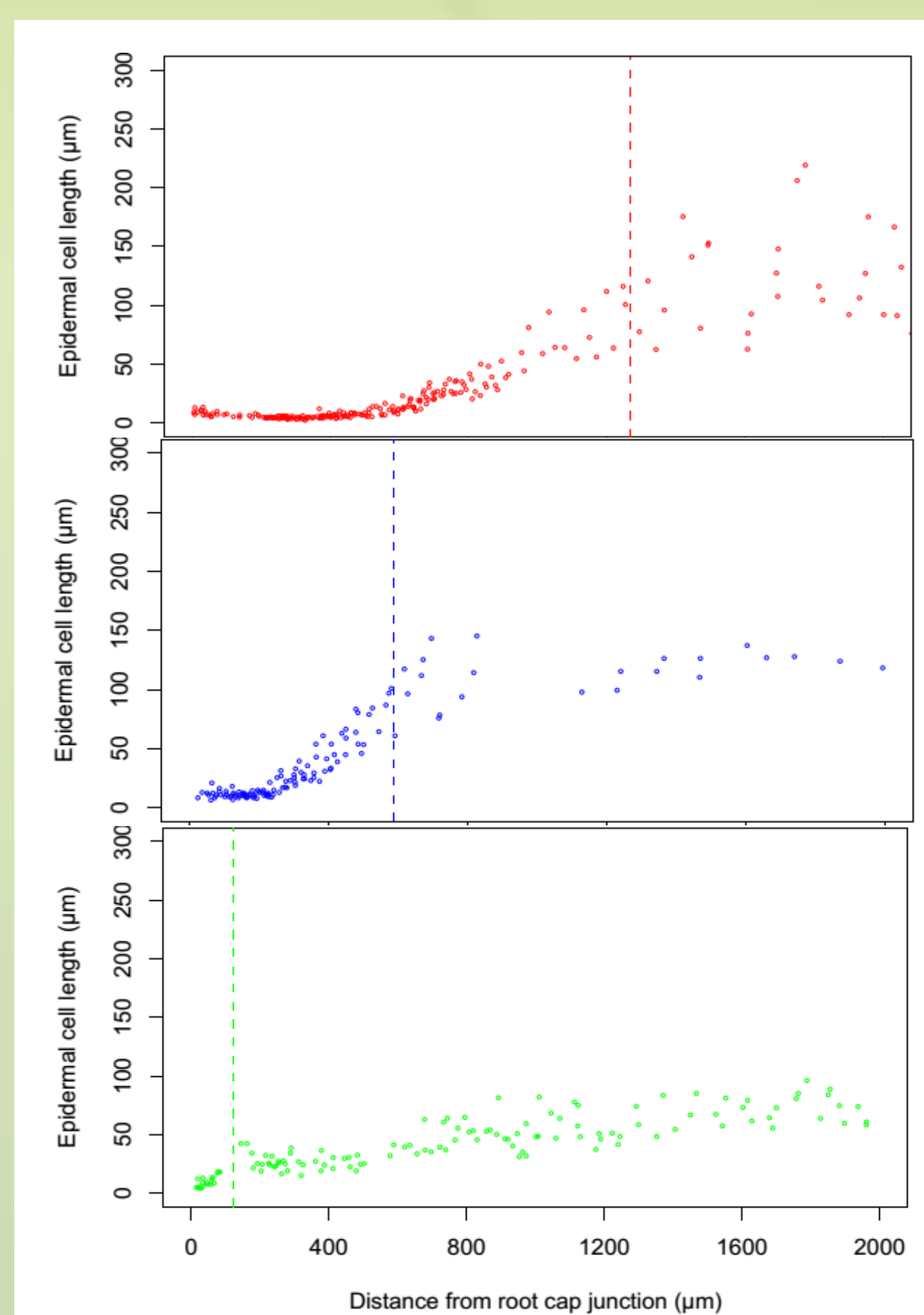
A first type of roots (called A) showed maintained elongation rate and apical diameter, giving rise to very long roots.

A second type of roots (B) showed gradually decreasing elongation rate and a progressive decrease in apical diameter.

A third type of roots (C) showed rapid cessation of elongation reaching a length < 10 mm associated with a rapid decrease in apical diameter.

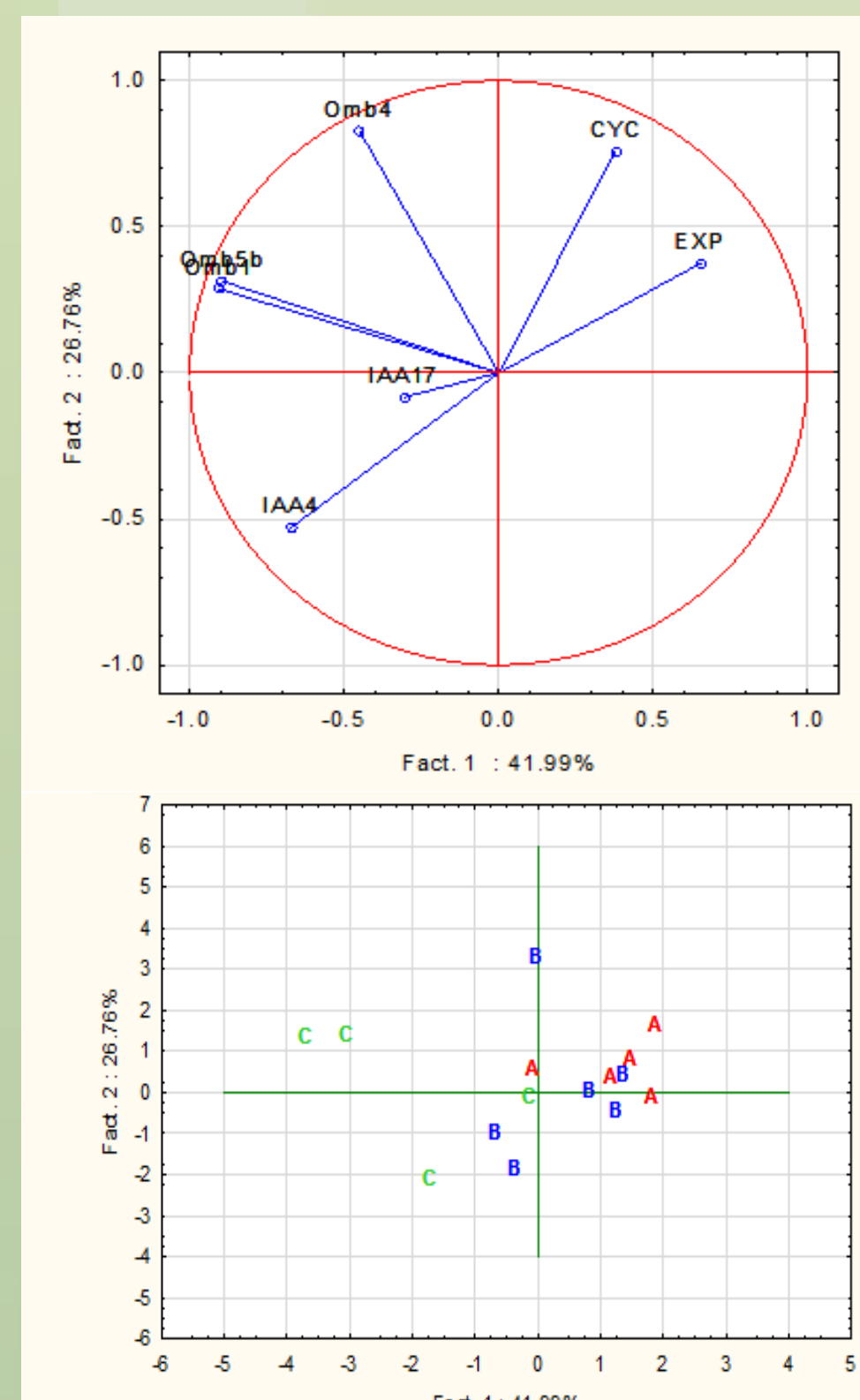


### 3 Cellular and molecular fingerprints associated with LR types



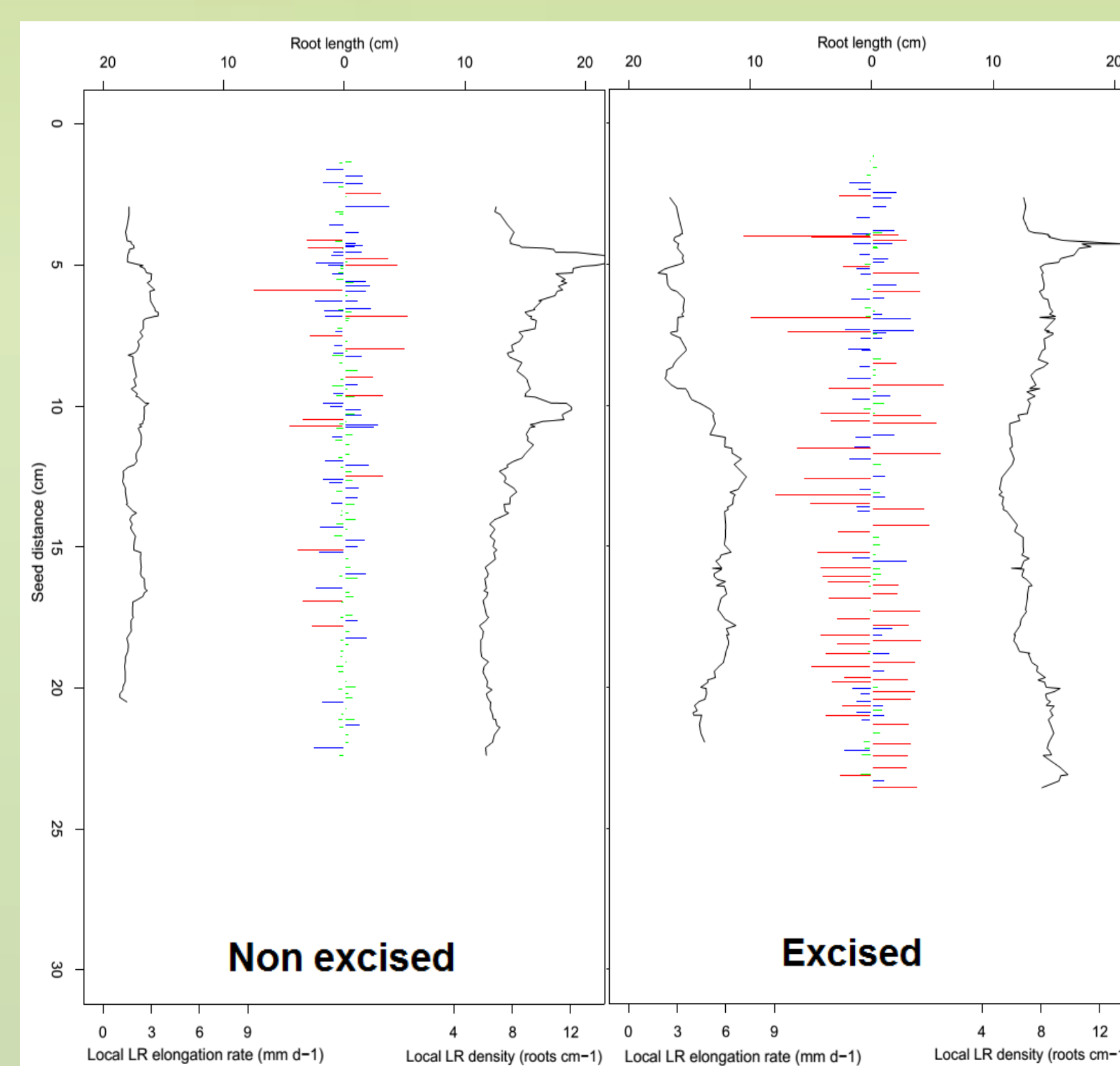
Cortical cell length patterns as a function of distance to root tip were clearly different in the 3 root classes with a much longer **meristematic zone** in A compared to B and almost a disappearance of meristem in C. The first root hair was closer to the tip in C than in A, B being intermediate.

Symbol	Gene name	Responsive to
EXP	B4 expansin	cellular elongation
CYC	cyclin	cellular mitosis
OMB1	asparagine synthase	carbon starvation
OMB4	vacuolar invertase	carbon starvation
OMB5.5b	sucrose synthase	carbon starvation
IAA4	unknown function	auxin abundance
IAA17	unknown function	auxin abundance



A set of 7 genes including genes responsive to **sugar starvation** and **auxin abundance** was used to associate root types with molecular fingerprints. A principal components analysis was used to build a molecular profile of each LR type. Type A roots have an elevated expression of CYC and EXP markers and low levels of auxin and sugar starvation genes. Type C roots have an opposite fingerprint, while B type roots have a pattern intermediate between A and C.

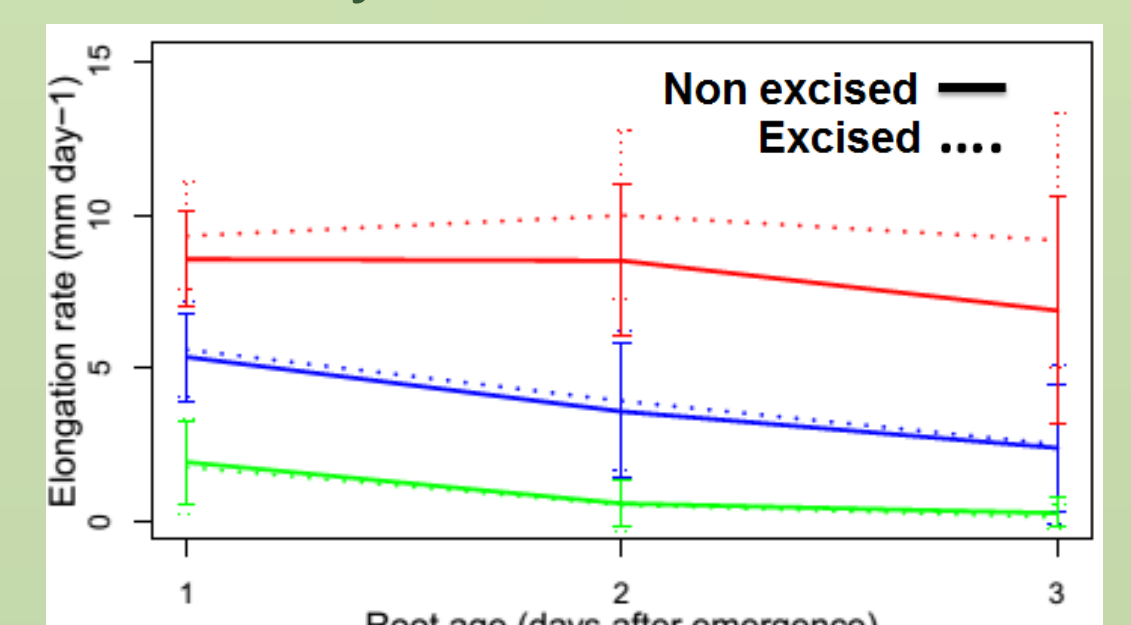
### 4 Auxin and sugars are involved in the variability of LRs



**Seminal and nodal root excision** was used to alter the fluxes of sugars and auxin from the shoot to the LRs present along the primary root.

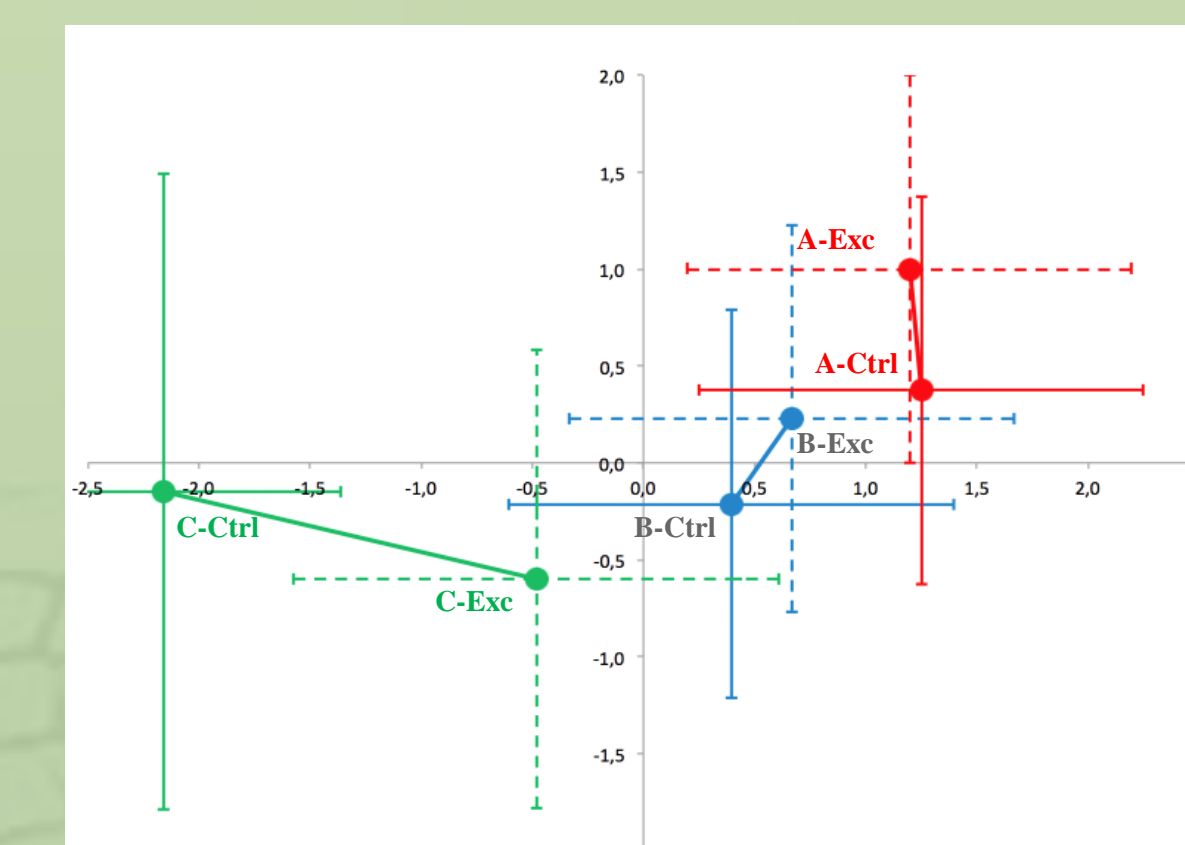
The excised root systems exhibited a **modified proportion of LR types**, with an increased number of type A roots and a reduced number of short C roots and a stimulated elongation rate for A roots only.

Treatment	A (%)	B (%)	C (%)	N plants	N roots
Control	16	38	46	3	509
Excision	32	36	32	3	528

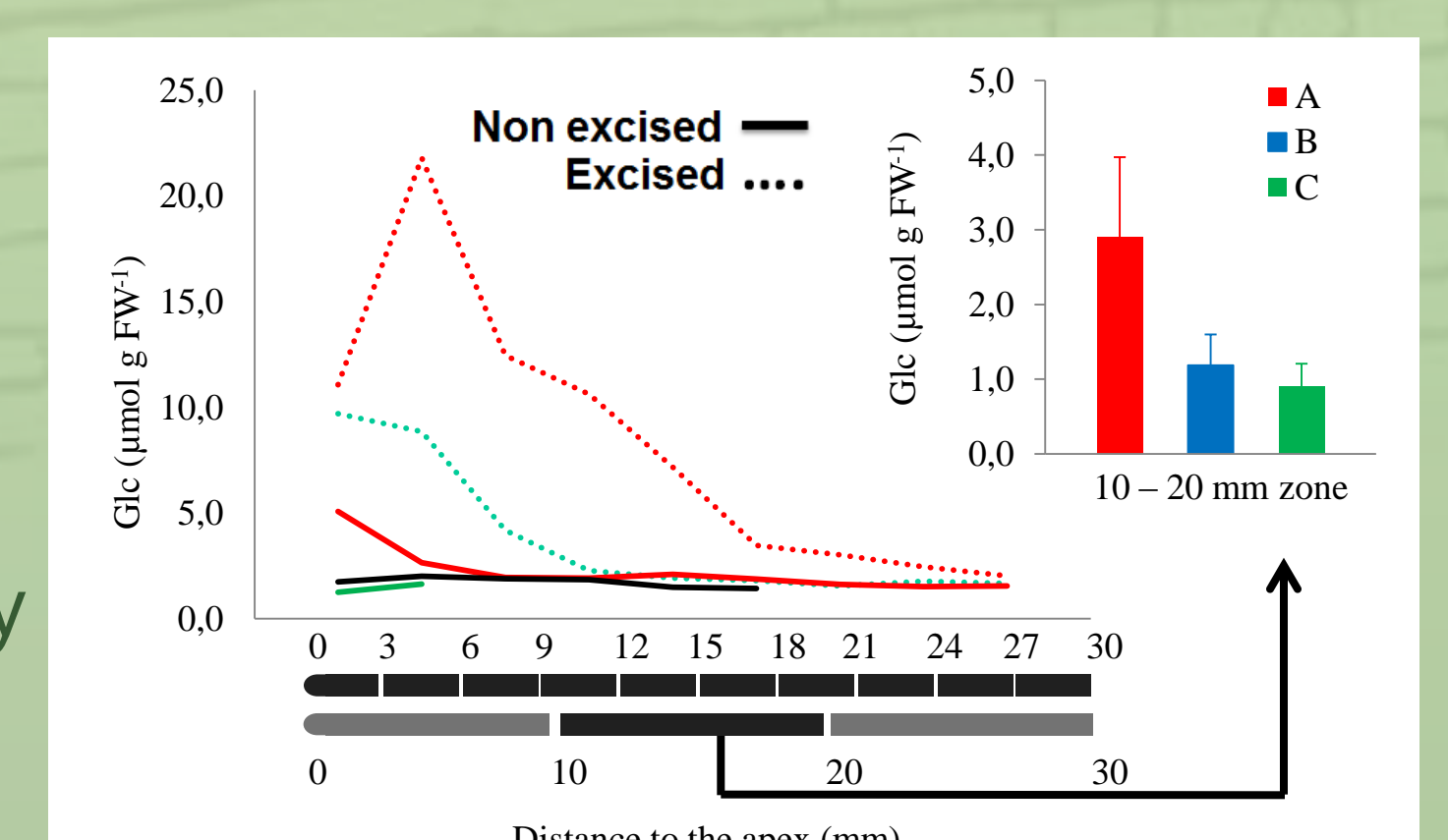


### 5 Conclusion and perspectives

- We have established a phenotyping and analysis pipeline able to extract data on single LR growth profiles and make them usable for in-depth statistical analysis.
- In maize, LRs can be classified in 3 contrasting types with sustained elongation and diameter (A), decreasing growth and diameter (B) and rapid cessation of elongation (C). Decelerating (B) and arrested (C) roots show reduced meristem size while arrested roots show sugar starvation.
- Excision of seminal and nodal roots massively modified sugar and probably auxin fluxes with consequences on both the proportion and the elongation rate of sustained growing roots (A).
- Our results support the hypothesis of a role of auxin and sugars in the control of the variability of LR growth.
- Additional experiments are needed to uncouple auxin from sugar fluxes, possibly using mutants or chemicals altering auxin transport.



Excision induced an **overflow of sugars** towards the tip of A and B roots as shown by the massive (x 20) increase of glucose concentration in the tip of these roots.



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